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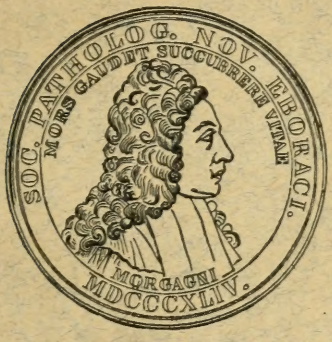

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Proceedings

of the

New York Pathological Society.

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DR. W. G. MACCALLUM, *President.*

A CASE OF ADENO-CARCINOMA OF THE CORPUS UTERI WITH PRONOUNCED METAPLASIA.

ROBERT T. FRANK, M.D.

The history showed that the patient had entered the menopause four years previously. Recently she again began to bleed, but irregularly. Curettings obtained demonstrated the presence of a carcinomatous growth. On opening the uterus a somewhat pedunculated growth, the size of a pigeon's egg, was found at

the fundus, midway between the tubal openings. Macroscopically the tumor appeared like the usual type of adeno-carcinoma located in this region. Microscopically, however, direct transitions from adeno-carcinomatous formations into squamous-celled complexes could be demonstrated. No reference to such a condition was found in the literature.

There are a few reports of the coincident appearance of adeno- and squamous-celled carcinoma of the body of the uterus. However, in most of these cases there was likewise a carcinoma of the cervix, and it appears very doubtful that the squamous-celled carcinoma of the body was primary. It is more likely that in these instances the corpus growth was metastatic or a direct extension of the cervical cancer. Kaufman reported a tumor of the uterine body in which adeno- and squamous-celled carcinoma occurred together, the cervix being unaffected.

The tumor shown to-night is primarily an adeno-carcinoma. The gland complexes invade the uterine muscle. Toward the surface a gradual and unbroken transition into squamous cells becomes evident. Some "pearl" formation is seen. The squamous cells evince no tendency to invade adjacent structures. This lack of invasive qualities combined with the localization to the less well-nourished surface portions have led me to interpret the findings as a metaplasia due to nutritional causes. No epidermization of the endometrium, sometimes noted after the menopause, was encountered in other parts of the uterus.

The second case has a two-fold interest: first, because of its rarity; second, because the condition was found at such an early stage. The case is one of

PSEUDO-MYXOMA PERITONEI, ARISING FROM THE VERMIFORM APPENDIX.

This was an accidental finding during a laparotomy performed to ventrofix the uterus and remove the appendix (which had caused vague symptoms referable to the right iliac region).

The cecum was found in its normal position. It was bound

down by velamentous adhesions. After these were separated, the appendix came into view behind the intestine. The appendix was found lying over the Psoas muscle, but raised away from the muscle by a thick circumscribed layer of gelatinous tissue which is best described in the words of Neumann, who reported a similar case, as a conglomeration resembling frog's spawn. The appendix and the surrounding jelly-like mass were removed in toto. Both ovaries and tubes appeared entirely normal.

The appendix macro- and microscopically proved normal. The jelly-like mass, on section, showed mucinous material, fibrin and goblet cells, such as are seen throughout the mucosa of the gastro-intestinal canal.

The cases found in the literature number only seven or eight. In all either a hernia of the mucosa or a rupture of the appendix was noted. Several recurrences are on record, in spite of the fact that all of the diseased tissue was apparently removed. Some remained permanently well after re-operation; others died of intestinal obstruction, infection, etc.

In the present case, though the outlook is slightly doubtful, the early time of operation warrants a somewhat more hopeful prognosis.

Discussion.

Dr. Mandlebaum said he recently had a case of carcinoma of the cervix uteri in which two distinct types of tumor were present, squamous as well as cylindrical. At that time, in searching through his material and getting together one hundred cases of carcinoma of the uterus, extending over a period of ten or twelve years, he found three cases of primary carcinoma of the corpus uteri, in which there was a distinct condition of metaplasia into the squamous-cell type. Although he had looked up the matter at that time, he could not find any good references in the literature as to the frequency of this condition, but in the three cases mentioned this type of metaplasia was undoubtedly present.

Dr. Frank asked Dr. Mandlebaum whether in his cases any

of the squamous cells were found to invade the deeper layers of the uterus.

In answer to Dr. Frank's question, Dr. Mandlebaum stated that, as far as he could recall, the change was simply on the surface. There did not seem to be any growth into the musculature of the uterus in these cases. He also stated that in one of his three cases there were distinct epithelial pearls present.

AN EXPERIMENTAL STUDY OF THE CAUSES WHICH PRODUCE THE GROWTH OF THE MAMMARY GLAND.

ROBERT T. FRANK, M.D., AND A. UNGER, M.D.

In all mammals hyperplasia of the breast takes place during pregnancy. Shortly after the birth of the young, milk secretion sets in. Ribbert transplanted the breast of a guinea pig to the root of its ear; milk secretion occurring in a subsequent puerperium proved that breast function does not depend upon the nervous system. Basch and others have demonstrated that the quantity and composition of the milk secreted is likewise independent of nervous stimuli.

The development of the breast at puberty is dependent upon ovarian function. This is proved by the non-development consequent to early castration. The pregnancy hyperplasia has been explained in several ways.

Halban claims that the placenta stimulates breast growth. He believes that when the placenta is expelled the breast begins to secrete. His hypothesis is based upon clinical observations. In abortion when the fetus dies, if the placenta becomes functionless, milk is secreted. If the placenta is retained and functionates, milk is not produced. In hydatid mole without fetus the breast hypertrophies, etc. The placenta is the antagonist of the ovary during pregnancy and inhibits its activity.

Starling's (1906) theory of breast hyperplasia is at present the most commonly accepted. Claypon and Starling injected virgin female rabbits daily with extract of rabbit fetuses. In six out of ten experiments a varying degree of breast growth was noted. The injections were made intraperitoneally, in order not to bathe the breast tissue with proteid solution. Extracts filtered through a Berkefeld candle and boiled extracts produced these effects. Extracts of the ovary, placenta and mucous membrane of the uterus were ineffective.

Starling concluded, from these experiments, that the breast hyperplasia of pregnancy was due to a highly diffusible chemical substance present in very small quantities throughout the body of the fetus and constantly taken up by the mother's blood. This *breast hormone,* like secretin, withstands boiling. It has a specific action upon the dormant breast acini and causes them to hypertrophy. At birth the hormone's anabolic influence is removed and the hyperplastic cells begin to secrete.

Foa (1908) injected rabbits with extracts of cow's fetuses. He found that unboiled extracts of heterologous fetuses produced the same growth as that reported by Starling, but boiled extracts were ineffective.

Biedel and Koenigstein (1910) repeated and extended Starling's work, also employing rabbits. They obtained hyperplasia with homologous extracts, and with entire fetuses implanted in the peritoneal cavity. In two experiments these investigators castrated the rabbits before beginning the injections without, however, affecting the positive results.

Finally, Basch (1910) published a new theory based upon experiments on rabbits, guinea pigs, dogs and children. According to his views, the combined secretion of ovary and placenta produce the hyperplasia of the breasts. Basch gives a few details and indicates merely by inference that the injections of the placental extract were made subcutaneously. Moreover, he used chiefly multiparous animals shortly after their milk secretion had ceased. His work merits no discussion.

Our own work was nearly completed before the reports of

Basch, and Biedel and Koenigstein were published. Foa's work was not known to us at the time we began our experiments.

The object of our research was primarily to ascertain whether heterologous fetal extracts produced breast hyperplasia, in order that we might use fetuses of a readily obtainable species. It would then be possible to use large quantities of extracts and perhaps by exaggeration of stimuli transform physiological into pathological growth. Furthermore, we determined to use rats, in order to be able to study the effect of tumors transplanted into an environment subjected to artificial and controllable stimulation.

Employing Starling's technic, we injected eight white rats with extracts of cows' fetuses. No increased development of the breasts occurred.

We then repeated the experiments, using rats' fetuses, ovaries and placentæ. The results were likewise negative.

Thereupon it became necessary to control Starling's results. Using his technic, we injected three series of rabbits with extracts of rabbits' fetuses, ovaries, placentæ and hypophyses. In all, 12 animals were used. Of these 5 received extract of fetuses, 2 respectively extracts of placentæ, extracts of ovaries, and extracts of hypophyses of pregnant animals, and 1 a combined extract of ovaries and hypophyses. In the last series rabbits previously castrated were used. Not only did the results vary greatly in each series, but also showed no correspondence to the tissues used for injection. For instance, one placental animal showed more hyperplasia than any of the fetus rabbits. Two ovarian animals showed marked growth, the other hardly any, etc. We were, therefore, forced to conclude that some as yet unknown factor must be accounted for.

To discover the cause of variation we, at intervals, removed breasts from normal untreated rabbits. It soon became apparent that at different periods a marked increase in size and of functional activity may occur in the breast of virgin rabbits. At any given period all the breasts are of the same size, but when re-

moved at successive intervals many animals show a progressive increase in size and in the number of ducts and acini.

As yet we have been unable to determine whether this change consists of a cyclical increase and decrease, such as was reported by Bouin and Ancel. In fact we have never noted a diminution in the size and extent of ducts, although regression of the acinous portions does seem to occur. The effect of castration, likewise, is not yet clearly proved. We have, however, found that no rapid involution follows removal of the ovaries.

Our specimens show that physiological hypertrophy accounts for the results obtained by Starling, Foa and Biedel and Koenigstein. The extreme differences obtained in our three series also demonstrate that the injections do not influence these physiological variations, otherwise some concordance would be noticeable. Consequently, we feel justified in concluding that Starling's theory of a fetal hormone has no basis of experimental proof.

The results of our work so far are purely destructive. We hope, however, in the near future to offer an explanation which is more in accord with the other phenomena of gestation. In the uterus certain well-understood changes take place at regular intervals, in order to prepare for a possible pregnancy. These changes are dependent upon the corpus lutem. There is some evidence that similar changes occur in the breast, and probably these, likewise, are effected by the yellow body. What factor, after impregnation, causes the persistence and exaggerated activity of the corpus lutem is quite unknown. Perhaps it is the fetus; but, if so, its action is indirect, and the necessarily crude method of injecting extract of fetuses offers little promise of solving the problem.

Dr. Frank then demonstrated lantern slides of the various forms of breasts which are found in rabbits and the changes obtained after injection of various extracts.

Discussion.

Dr. MacCallum said it seemed that the effect which Dr. Frank had shown in the menstrual changes, as bringing about

difference in the size of the breasts, is extremely important and made it seem at once that this might be the explanation of the results which Starling had ascribed to his injections. He thought that Dr. Frank would surely agree, however, that there must be something in the way of a chemical substance possibly existing during the course of the menstrual changes, which acts very much more intensely during the course of pregnancy; but that this substance was not demonstrated by the injection experiments of Starling.

Under all circumstances the injection of a substance day by day seemed a very poor substitute for the gradual secretion of the living organs, and it was only in connection with the thyroid that any real approach to the functions of the organs of internal secretion had been attained by the injection of extracts.

A SARCOMA TRANSMITTED BY AN AGENT SEPARABLE FROM THE TUMOR CELLS.

PEYTON ROUS, M.D.

The decade of work with transmissible neoplasms has much increased our knowledge of tumor behavior, and has disclosed the existence of immunity processes directed against such growths; but it has scarcely affected the problem of tumor etiology. All investigations with the neoplasms of the rat, mouse and dog have failed to bring to light their cause, and they can still be transmitted only by transplanting the living cells. The present instance is the first in which it has proved possible to separate from tumor cells an agent that will cause the disease in new hosts. Since the importance of the observation depends entirely on the characters of the growth employed, these will be discussed in detail.

Description of the Tumor.

(Lantern slides and charts were shown in illustration.)

The original growth occurred in the subcutaneous tissue of an otherwise healthy chicken. The attempt to transplant it proved successful and the tumor has thus far been propagated in eleven consecutive series of fowls. Histologically it consists of spindle cells, arranged in bundles, and supported by a slight vascular framework. By means of special stains intercellular connective tissue fibrils can be demonstrated; and mucinous ground substance is sometimes found. As a rule the cells have a well-defined spindle shape, but at the edge of the growing mass they may be ovoid or polyhedral, and only later take up the spindle form. The cell nucleus is vesicular, and relatively large. Division is mostly by amitosis, though mitoses are frequent. Although the growth has been transplanted often, the morphological changes in it have been relatively slight. Microscopically it is always a spindle-celled sarcoma or myxosarcoma.

In the gross the tumor is usually firm and gristly, but it may be friable, or even gelatinous, containing in the last instance much true mucin. The color of the neoplastic tissue is grayish or yellowish white, and the cut surface finely striated. Enlargement of the mass takes place both by expansion and by active invasion and destruction of the surrounding tissues. At its center coagulation necrosis, cyst formation or hemorrhage are commonly seen. The health of the host is at first unaffected, but, with the continued growth of the neoplasm, emaciation sets in, and the fowl becomes cold, weak and, for a day or two before death, comatose.

The growth which results from an implantation of the neoplastic tissue may remain local, but more frequently metastasis takes place from it. At autopsy the lungs may be almost replaced by discrete gristly nodules which are found also in the liver, heart, and to a less extent in the other viscera. The blood-stream is the usual path of dissemination, though it may follow the lymphatics. Contact metastasis, and a scattering of the growth in the serous cavities are both frequent.

It has been of importance to determine how metastasis takes place. The histological evidence for an origin of the secondary nodules through lodgement and growth of cells detached from the primary mass is more conclusive than with most tumors. Many instances are met with in which a strand of the neoplastic tissue has penetrated one of the thin-walled blood vessels and proliferated within its lumen. Such a strand, bare of endothelium, may extend for a considerable distance in the blood stream. A study of early metastases indicates that they arise by growth from a small vessel occluded with tumor tissue. The distribution of the metastases is also such as would indicate cell-transportation, namely, to the lungs first, later to the viscera supplied by the systemic circulation. And the transplantability of the cells has been proved by a microscopic examination of grafts of the tumor tissue removed shortly after implantation.

The fate of tumor grafts in the susceptible and immune host is as follows: The bit of neoplastic tissue is vascularized within three to five days after its introduction into a susceptible fowl, and its constituent neoplastic cells survive, and proliferate, giving rise, it would seem, to all of the resulting mass. There has taken place a real transplantation of tissue. In a resistant host, on the other hand, either vascularization does not ensue, and the graft dies from lack of nourishment; or, following vascularization and transient growth, the tumor elements succumb in the midst of an accumulation of small, round cells histologically identical with lymphocytes. The second process is the one observed during the spontaneous retrogression of large growths, which occurs not uncommonly. The dwindling mass of living tumor cells is surrounded by a zone of fibrous connective tissue and is invaded here and there by lymphocytes. All these phenomena are similar to those described for the neoplasms of mammals.

Dr. James B. Murphy and I have recently transplanted the growth into developing hens' eggs, by means of a little window in the shell, which is afterwards sealed. In the embryo, or its membranes, the neoplastic cells proliferate rapidly and form

within the course of a week relatively large discrete nodules. Under the microscope these consist of the characteristic spindle cells, growing in a diffuse mass, unsurrounded by cellular reaction in the host tissues. Indeed, in the susceptible adult host there is a striking absence of cellular reaction about the growing tumor.

Behavior of the Growth on Transplantation.

The original growth occurred in a pure-bred Plymouth Rock hen, and its transplantation was accomplished by the use of other individuals of the same stock. Bits of the growth were implanted in the breast muscle by means of a trochar—a procedure now the routine. For a long time the tumor could only be propagated in fowls of the variety in which it first occurred, and even yet they are much the best hosts. Repeated inoculations have been made in birds of other species and in mammals, but always with negative results. Although susceptible normal chickens and others with the tumor have been kept together in close quarters for long periods, no instance suggesting a natural infectivity of the disease has occurred.

Repeated transplantation has produced other changes in the neoplasm besides that of overcoming the specificity which at first limited its further transmission. The rate of its growth has much increased; it develops in a larger percentage of fowls of the susceptible variety; and it has acquired the power to metastasize. The growth rate is now extraordinarily rapid. A great bulging mass reaching from clavicle to groin, completely replacing the muscle and much of the subcutaneous tissue, may form in the course of three weeks after the implantation of a graft two millimeters in diameter. A majority of the living cells in such a growth will be found in process of division. Metastasis at first did not occur. Then it appeared late, infrequently, and was limited to a few organs. But now it is rarely absent in fowls dying of the disease, takes place early, and may involve many of the viscera.

Young individuals show themselves more susceptible as hosts for the transplanted growth than do old ones—a fact well known of the sarcomata of rats and mice. Until the tumor has reached a diameter of three to four centimeters, it is singularly dependent for its success on the good physical condition of the host. An intercurrent emaciating illness may entirely check its growth or even cause it to disappear, though should the fowl later recover health the tumor may return and grow rapidly. Some of the tumors of mammals exhibit a similar striking dependence on the condition of the host.

Several types of resistance to the chicken tumor have been observed, and they are similar to those already known for the transmissible neoplasms of other creatures. Reference has already been made to a resistance based on the fowl's race, and one dependent on its age. A natural resistance is met with in some young fowls of the susceptible stock. The individuals manifesting it can be inoculated again and again without success. Finally there should be mentioned an acquired resistance possessed by healthy fowls in which a tumor has retrogressed, protecting them against growth from subsequent inoculations.

The Nature of the Tumor.

Histologically the growth is a sarcoma and all the features of its behavior identify it as a malignant neoplasm—of which, indeed, it may be taken as a type instance. Our present conception of the typical malignant neoplasm is a composite derived from clinical observation, morphological findings, and the results of transmission experiments with the tumors of animals. No growth which runs its course in a single individual can yield all the features of the composite. For, although such a growth may be histologically characteristic, and perhaps metastasizes and produces a cachexia in the host, yet there exist no data as to whether, on transplantation, it would have exhibited the further features of a neoplasm. While the transmissible tumors are easily tested on these points, they, on the other hand, often lack

in more obvious features, failing to infiltrate or to metastasize. It thus happens that of the many destructive neoplasms met with, comparatively few, and these of necessity transplantable, can correspond at all points with the type picture. Among them may be mentioned the Lewin carcinoma, the Flexner-Jobling carcinoma, and the sarcoma of the chicken here described.

Separation of an Agent Causing the Growth.

Much of this part of the work has been done in association with Dr. James B. Murphy.

From a consideration of the histological features of the sarcoma the impression was obtained that its growth is strictly dependent on the proliferation of cells already neoplastic. There was no reason to suppose that its transmission could be accomplished in another way than by a transplantation of the cells. On this account no attempt was made to separate out an etiological agent from the growth until the increase in its malignancy had brought what seemed to be an exceptionably favorable opportunity. It was first found that the sarcoma could be produced in new hosts by the injection of fluid obtained on passing through ordinary filter paper a suspension of the ground tumor in salt solution. The fluid supernatant after long centrifugalization of a suspension also caused tumors. Resort was then had to Berkefeld filters tested under the conditions of the experiment and found to hold back *Bacillus prodigiosus*. Using a three or four per cent. suspension of the tumor in warm Ringer's solution several different filtrations were made. Relatively small amounts of the resulting fluid (0.2 to 0.5 cc.) yielded tumors in some of the susceptible hosts inoculated; in one instance where larger amounts were used (0.5 to 1.0 cc.) eighty per cent. of the fowls developed growths. The sarcomatous tissue when dried over sulphuric acid *in vacuo* retains its ability to cause the growth in new hosts. The characteristic tumor has been obtained by an inoculation of material dried to a powder and kept for twenty-seven days at room temperature. Two attempts to pass through

a Chamberland filter the agent causing the growth have been unsuccessful, but neither will withstand criticism on the technical side. The mucinous constituents of the growth even when much diluted rapidly clog such filters.

The growths which result from the injection of dried tissue or of a Berkefeld filtrate do not differ histologically from such as follow implantation of the living sarcoma cells, but they are late in appearing and a majority of them grow slowly. Many eventually metastasize, nevertheless, and lead to the death of the host. Tumors caused by the injection of a Berkefeld filtrate will themselves furnish material capable of causing tumors after suspension and filtration.

Character of the Agent.

Frequent attempts have been made to observe the agent causing the growth. Cultures from the living tissue remain sterile, so far as can be seen, even when made upon chicken blood-agar or upon media containing the unheated constituents of Plymouth Rock eggs. Stains, and the examination of fresh smears or of the filtrate with the dark-field microscope have likewise yielded a negative result. Whatever the causative agent, it is at or beyond the limits of visibility with the microscope. To conclude that we have to deal with a self-perpetuating parasitic organism is natural; but the facts can be well explained by supposing a chemical stimulant elaborated by the neoplastic cells, and capable of producing the disease in a new host, with, in consequence, a further elaboration of the same specific stimulant. However, no example can be cited in support of this hypothesis, whereas there are a number of diseases known to be caused by ultramicroscopic organisms.

Significance of the Findings.

The ultimate significance of these findings is hardly to be discussed at present. Perhaps all sarcomata, and even all carcinomata, will be found to depend, like the chicken tumor, on

a causative agent separable from the cells. Or perhaps this tumor will remain unique in its etiology. Only the facts can decide. But there is an immediate significance to the findings that is obvious and considerable. Many of the characters of the malignant neoplasm have been taken to show that such diseases are dependent on disordered cell-metabolism, and while precipitated by external factors often, are otherwise independent of them. Yet here is a growth with just such characters, which can be produced in healthy individuals by the action of an agent derived from the neoplastic cells, in a sense self-perpetuating, and not improbably parasitic. It follows that the behavior of malign tumors can no longer be cited as evidence of their dependence on an intrinsic cell-derangement.

Discussion.

Dr. Beebe: These experiments are extremely interesting. I think we are not yet in a position to say what they mean. The fact that many experiments of this character have been tried with the other types of transplantable tumors, namely, those in rats, mice and dogs and have never given growth indicates that this tumor in the fowl stands in a class by itself. I believe that reports have appeared showing that the leukemia in fowls which is readily transferred may also be transmitted by cell-free filtrates. We are evidently dealing with a pathological process which is unique in character. It may be that the technique which has been employed in these other experiments has not been of the right type. Possibly the temperature or the reaction of the suspended medium has not been precisely right to preserve what may prove to be a fairly delicate mechanism in transmitting these growths, at least in cell-free filtrates.

If one is very strongly inclined toward the parasitic theory, it is possible to find in these experiments a satisfactory explanation of the process. It need hardly be said that such a position must be taken with a vast deal of care, for this tumor is unique in many respects, and as far as the experimental tumors are

concerned, we have nothing that exactly corresponds with it in other species of animals. One of the most striking differences is the fact that this tumor shows metastases very early in its course while the tumors transplanted in other animals mestastasize comparatively late in their development.

I should be very much interested to know more about this matter than Dr. Rous has stated, probably more than he is now able or willing to say; for instance, the relation of temperature is one of importance. To what degree may these extracts be heated or cooled before the peculiar qualities are killed? Would it be possible to give inoculations of such heated or cooled extracts that in themselves would not produce tumor growth and yet that in themselves would not produce tumor growth and yet would produce immunity to a subsequent implantation of living tissue. The experiments are most interesting and suggestive and will stimulate cancer research in allied animal forms.

As regards the etiology of tumors in general, we are not yet able to do more than quote the facts that have been produced and not proceed too quickly to new theories.

Dr. I. Levin: I wish to say a few words in indirect support of Dr. Rous' work. I applied the exact techique of Dr. Rous' to a transplantable sarcoma of the white rat, and the results were uniformly negative. Consequently my filtrate of the sarcoma did not contain any cells and I may infer that Rous' filtrate does not contain any cells either. Apparently the tumor with which the Doctor is dealing may be propagated without the aid of the cells of the primary growth.

But the point that needs further corroboration is whether Dr. Rous induces in the new host the formation of a true metastasizing sarcoma or of multiple granulomata. I have shown a couple of years ago, that it is extremely difficult to differentiate these two conditions morphologically. It would be of great importance to investigate whether by some chemical or mechanical stimuli one could not induce the formation of similar multiple granulomata in the fowl.

Dr. Field: I would like to ask Dr. Rous some questions in

regard to his use of filters. I have had a good deal of experience dealing with filters of one sort and another and I have not that faith in them that I had when I first started.

As I understood Dr. Rous, he made but one test as to the permeability of these filters and that was with regard to their preventing bacillus prodigiosus from passing through. Now, that is an exceeding large organism and a filter that might not allow the passage of this organism might be permeable to other organisms and especially to cells showing any degree of ameboid motion.

Another point, how long was the fluid in contact with the filter? In other words, how much time was allowed to pass the fluid through the wall of the filter, also under what pressure? It seems to me that these points should be thoroughly investigated before one draws any conclusions whatever. We all know that the mere passage of large quantities of fluids through any type of filter does not render them absolutely sterile though it certainly leaves only a minimum number of cells, and in regard to this point the comparative number of takes in injected fowl with the filtered and unfiltered materials ought to give one a clue.

Dr. MacCallum said that Dr. Rous' work seemed to have an especially striking relation to the various ideas which have been expressed in recent years as to the parasitic nature of tumor growths. Inasmuch as certain infectious diseases seem to depend upon an organism which, in some of its stages can be passed through a Berkefeld filter, the suggestiveness of the results is very striking.

It would be interesting, possibly, if Dr. Rous would attempt the stimulation of the growth of chicken tissue in vitro by the addition of some of this filtrate to the plasmatic media.

A CASE OF GASTRIC TETANY.*

DR. LARKIN.

Dr. Larkin expressed regret at not being able to present a complete record of the case as he had performed the autopsy the day before.

The patient was a hostler, forty-four years of age, and was taken ill two days before death. While occupied in a stable, he was seized with a sharp pain in the abdomen and when help arrived, was found stretched on the floor absolutely rigid. His head could be moved but the patient could not move his legs. He was semi-conscious. After three-quarters of an hour, the stiffness of the legs and arms relaxed somewhat. The patient refused to be taken to the hospital and remained in the stable until four or five o'clock in the afternoon when he was seized with another sharp pain in his stomach, vomited and passed again into a condition of general rigidity; his arms being gradually drawn up over his head and his legs becoming spastic. About eight o'clock he was again taken with a similar seizure, the third within a period of twelve hours, and in that condition was removed to St. Vincent's Hospital.

Dr. Stewart made the diagnosis of gastric tetany. Patient was pulseless, had very slow respiration, suffering from a great deal of respiratory oppression. After his entry into the hospital the period of general spasticity of his abdomen and extremities was at times accompanied by severe pain. After four hours in the hospital, he again relaxed and during this period of relaxation his pulse became almost normal, the temperature sub-normal. The abdominal distress persisted during this period of relaxation; he became able to speak, but not intelligently, so that they were unable to obtain from him an accurate statement as to the previous duration of his illness. After six hours in the hospital, he was again seized with another and fatal attack, accompanied by great respiratory depression. After an hour and a quarter, there being no relaxation of the muscles, he died apparently from asphyxia.

*Presented at the January meeting of the N. Y. Pathological Society.

The autopsy showed a condition perhaps familiar to only some of the persons present. The stomach contained 4½ liters of fluid. The musculature of the stomach is very much thickened, especially along the anterior border, although there is no evidence of change in the mucous membrane. This thickening was not due entirely to oedema but rather to muscular hypertrophy and increased connective tissue growth in the sub-mucosa. At the pyloric end of the stomach, there was an old ulcer which had led to very marked constriction of the pyloric opening. The oesphagus was longer than normal and the position of the stomach in the body was not the normal one. Adhesions to the transverse colon and a long band from the great omentum to the tip of the bladder pulled the stomach over from its ordinary position. The parathyroid glands in this case had not yet been dissected out and Dr. Larkin said that if they were found to show lesions, he would report upon them at a later date.

PRIMARY LYMPHOSARCOMA OF THE STOMACH.

DR. LARKIN.

Dr. Larkin presented a case of lymphosarcoma of the stomach with lymphocytosis. The specimen was from a man fifty-four years of age, who had been under observation for a period of two months. Previous history was that of secondary anaemia, which had been progressive. There had been numerous blood examinations and before entering the Post-Graduate Hospital, the diagnosis of Leukaemia had been made, but was subsequently nullified. The symptoms at that time were those of an advanced secondary anaemia. The first evidence of local disease was a sharp pain in the epigrastric region, which from time to time grew worse, but there was no vomiting throughout the illness. About ten days before death, which occurred rather suddenly, a small lump appeared in the neck and another lump in the inguinal region on the left side. The glandular enlargement in the

neck was removed and showed microscopically an ordinary lymphatic hyperplasia.

After having been in the hospital two months, the patient while going to the lavatory was seized with sharp pain. The next morning the abdomen became markedly distended. There was frequent vomiting and watery stools which were tinged with fresh blood. He died twelve hours after the beginning of his sharp pain.

The specimen presented is unusual. Looking into the stomach, one finds a very large ulcer which is perforated posteriorly into the lesser peritoneal sac. Along the greater curvature of the stomach there is found a flatter ulceration, the base of which is hard and about which the mucous membrane is raised. There are other depressions further back, as well as some smaller masses. If one compares the anterior border of the stomach with this large area, one finds great thickening of the mucous membrane. There is adhesion of the posterior part of the stomach with the pancreas which is equally involved in the growth. There is enlargement of the post-aortic lymphatic chain as far as the pelvis, resulting in compression of the aorta. The spleen is the seat of numerous smaller and larger masses, some of which have begun to undergo necrosis. The mass in the pelvis, along the iliac vessels, gives the general picture of lymphoma or lymphosarcoma.

The character of the lymphnodes, stomach, metastasis in the liver and the tumor itself is identical microscopically, the predominating type of cell being the lymphocytic; but one finds also some pyramidal cells and, in places, a large amount of hyaline stroma. The specimen is of some rarity, because of the position of the tumor.

DISSECTING ANEURISM OF THE AORTA.

CYRUS FIELD, M.D.

Dr. Field presented a specimen from a case of a colored man, 68 years of age, who entered Bellevue Hospital in the service of Dr. Alexander Lambert and died with a diagnosis of broncho-pneumonia and probable malignant endocarditis. The interesting feature of the case relates to the aorta.

On opening what was first taken to be the aorta, it was found that one entered a large arterial trunk lying to the right of the true aorta, and running parallel with it to its bifurcation. It was thought at first that one might be dealing with a case of double aorta; but further dissection showed that it was a dissecting aneurism. At a point in the aorta 2 cm. below the right subclavian, there is a circular opening 2 cm. in diameter in the aortic wall, leading into an aneurysmal tube, the internal surface of which is perfectly smooth and glistening, and which has the appearance of a large arterial trunk. Passing downward, the tube divides and follows both common iliacs, and the external iliacs beyond the inguinal canal. The right renal artery is given off from the true aorta; as it passes through the aneurysmal sac a portion of its wall has been eroded, so that there is an opening from the sac into this renal artery. The right kidney, therefore, derives its blood from both the circulation in the aneurysmal sac and the circulation in the aorta. That there was a free circulation within the aneurysmal system is shown by the absence of clot either in the main trunk of the aneurysmal sac or in any of its branches.

During life the patient gave no symptoms referable to such condition. It is interesting to note that in this case the Wasserman reaction was negative.

Discussion.

Dr. MacCallum showed some drawings to illustrate dissecting aneurisms, one of which was very similar indeed to the one shown by Dr. Field.

PRESENTATION OF SPECIMENS.

J. H. LARKIN, M.D.

Lipoma of Pleural Cavity.

I have two interesting specimens to present to the Society. The first one is a rather unusual case of tumor of the pleural cavity, a Lipoma. The patient was a man about 54 years of age, who had a number of sinuses around the rectum for which he had been operated on several times—in the Mount Sinai Hospital, in the New York Hospital and in Bellevue Hospital; he finally arrived at St. Francis Hospital, where he was operated on again and where he remained for a period of about two months. There were a number of deep sinuses and old scars of former operations, and with a probe one could pass readily down through the sinus far into the glutei, so that there was a constant absorption of pus and a constant febrile temperature for the period during which he was in the hospital. He finally grew markedly emaciated and died. The condition at the time of autopsy was rather interesting. After removing the chest wall it was found that the left lung overlapped the right, so that the entire anterior mediastinum could not be seen. Thinking that perhaps fluid had pressed the lung over on the left side, I put my hand down, and in doing so found no fluid, but a tumor which lay in the pleural cavity.

The tumor after hardening does not present the size that it did on removal. It is a lobulated mass, which flanges over the diaphragm along the line of its attachment to the ribs. The appearance is typically that of a lipoma, and this diagnosis is confirmed by microscopic examination. It does not, however, give the appearance of ordinary fat, but is a more solid mass, the capsule of which is traversed by small blood vessels.

The tumor is not so very interesting as a lipoma, because lipomata are of frequent occurrence. My experience with lipomata in general has been limited mostly to those occurring within the abdominal cavity. I have records of two or three lipomata in the gastro-colic omenum. They are very large and cause a

great deal of disturbance. But I have at least, in looking over my notes, two cases of lipomata of the retroperitoneal region around the aorta in the neighborhood of the cœliac axis. In going over the literature of tumors of the diaphragm, I have been able to find the record of but one case of lipoma of the diaphragm. That is a case which was published by Stricker in a small memorial, which was afterwards published in one of the early editions of Virchow's Archives. That is the only reference I can find of a lipoma of the diaphragm. In fact, in looking over the literature for tumors of the diaphragm, one is amazed at the rarity of tumors in this part of the body. The tumor that is reported by Stricker is more of a microscopic find. He gives the dimensions of the tumor as measuring about 2 cm. in diameter. The microscopic examination proved it to be a strictly fatty tumor.

A Case of Ludwig's Angina.

This specimen was taken from a doctor on one of the Italian liners. He was a man of about 155 pounds, and was said by his brother surgeons to have been perfectly well until about three days after leaving the Italian shore, when he began to have sore throat. This condition became rapidly worse, in spite of treatment. They had noticed that there was œdema of the glottis and of the soft tissues of the pharnyx; and after four days the condition of the individual had become so serious that tracheotomy was performed; this seemed to relieve him at first, but with the general swelling of the neck region and anterior part of the chest the respiration became markedly embarrassed, so that the tracheotomy seemed to fail. They then removed the tracheotomy tube and passed a long tube down to the bifurcation of the bronchi in the hope of giving him more air, and that seemed to relieve him more and more. As they were nearing New York the individual commenced to get more markedly disturbed, the œdema increased, and he died just as he was coming into the harbor.

At the autopsy the contour of the neck was entirely ob-

secured, so great was the swelling. The swelling came up to the ears and œdematous, brawny indurated masses extended from what appeared to be his jaw down to both shoulders, stopping short just at the deltoid muscle, so that the contrast between his head and the upper part of his chest was truly remarkable. The specimen which I present here to-night was obtained with a great deal of difficulty; one can see that the tissues show more or less brawny induration. No foci of pus were present; even when we dissected well into the axilla there was no suppuration. There was simply a deep, brawny, hard indurated tissue. There is not so much exudative inflammation as one would expect to find in such a dire condition. There is a still persisting œdema of the glottis mostly on one side, but acting as a valve.

The specimen here presents all the evidences of an ordinary diffuse cellulitis of the upper part of the neck. The bacteriology of this specimen does not give us any definite clue. The ordinary streptococcus has been isolated from this specimen in pure culture. The etiology of the condition is somewhat mixed up and no particular organism has as yet been associated with this particular lesion. The specimen is rather an unusual one on account of the tremendous amount of swelling and also on account of the condition of diffuse cellular infiltration. The microscopic examination of parts of this tissue here shows the ordinary evidences of a diffuse cellulitis of the neck.

Discussion.

Dr. Pappenheimer asked Dr. Larkin, in reference to the first case presented, whether the fat cells were of the adult or embryonic type?

Dr. Larkin replied that they were mostly of the small embryonic type and not adult fat cells.

TWO CASES OF CARDIAC ANOMALIES.

OTTO SCHULTZE, M.D.

Cor triloculare bi-auriculare: Specimen was obtained from a child about four months old, of whom no history could be obtained. The heart has two auricles, which are completely separated one from the other, and a single ventricle giving origin to aorta and pulmonary artery, which are separated by a short septum derived from the one dividing the common arterial trunk into aorta and pulmonary artery. The lumen of the aorta is larger than that of the pulmonary artery. The brain was almost completely anemic and the lungs congested.

Cor triloculare bi-ventriculare: This specimen was obtained from a female, 22 years of age; 55 inches in length, weighing probably 75 pounds. She was well developed, and had a perfect form, with the exception that the lower limbs were of the male type. When the knees were brought together there was a larger space between the thighs and calves than in the normal female. There was hair upon the cheeks and a few upon the upper lip. Hair in axilla was very scanty. Hair on legs and about peritoneum and arms. Contour of neck and shoulders rectangular. Shoulders were small and rounded.

At autopsy the heart was found completely contracted. Trachea and bronchi were filled with blood. The source of the hemorrhage was traced to the right lung, on sectioning which the superior branch of the pulmonary artery, over one-half inch in width, presented a parietal thrombus with open lumen extending as far as the main trunk. The pulmonary artery and its branches were greatly enlarged—nearly three times the size of the aorta—and at the entrance to each lung there were large aneurysmal distentions which extended into the smaller branches. The main trunk of the pulmonary presented a thin wall, the branches numerous plaques yellowish in color and thickened, without infiltration of lime salts. The hemorrhage had evidently proceeded from the rupture of a branch of the pulmonary artery into the right bronchus. The heart was very much enlarged, the right

ventricle and auricle being over three times the size of the left ventricle and auricle. There is a single auricle into which open superior and inferior vena cava, coronary sinus and right and left pulmonary veins. There is an indication of a septum between the mitral and tricuspid orifices.

The history of the case is as follows. The deceased was perfectly well, with the exception of dyspnœa on exertion. On leaving for work in the morning, she collapsed in the areaway of her home, with a copious hemorrhage from the mouth. It was stated that a year or so ago, while living in Chicago, she had been examined by a large number of doctors in a clinic, who told her that she had a musical heart.

STUDIES ON IMMUNITY IN CANCERS OF THE WHITE RAT; THE SIGNIFICANCE OF ATHREPSIA.*

I. LEVIN, M.D., AND M. J. SITTENFIELD, M.D.

(From the Department of Pathology of Columbia University.)

In a previous communication one of the present writers[1] indicated the fact that the result of tumor implantation is determined by two factors: first, whether or not the implanted tumor becomes ingrafted; and, second, on the rate of its growth if ingrafted.

The mechanism of the ingrafting is common to tumor and to normal tissue. On the other hand, the ultimate fate and character of the graft depends on the rate of growth of the implanted tissue.

The rate of growth of an implanted tumor and with it the ultimate character of the tumor depends upon an interaction between the power of proliferation of the tumor cell and the

*Conducted at the expense of the Geo. Crocker Special Research Fund.

[1]Levin, I.: *Journ. Exp. Med.*, 1916, xii, 594.

resistance of the host. The mere grafting of the tumor is independent of the phenomenon of cancer immunity.

A great deal of confusion and controversy that exists in the attempted analysis of the results of the work on cancer immunity is caused by this lack of appreciation of the co-existence of the two factors determining the outcome of the tumor implantation. It seemed, therefore, desirable to undertake a revision of the work on cancer immunity.

The most popular and most generally accepted theory of cancer immunity is the one advanced by Ehrlich and commonly designated the *athrepsia* hypothesis. It is based on the following considerations:

Immunity or resistance of the host to the growth of an inoculable cancer is *not specific* in its character, and an animal immune against carcinoma will be also immune against sarcoma. The methods used to induce an artificial immunization are, likewise, not specific. When a cancer of low virulence is inoculated into and absorbed by the host, the latter is immune in the majority of cases against subsequent inoculation of any of the most virulent cancers. This non-specific character of cancer immunity Ehrlich designates by the name of *panimmunity*.

According to Ehrlich, as well as most of the investigators on the subject, an artificial immunity may be induced only by treatment with living cells. Growth of inoculated cancers, on the other hand, is also due to cell activity. Ehrlich contends that the growth of a cancer indicates that the tumor cells possess a great avidity for a certain specific food within the organism of the host. When cancer fails to grow, then either the organism of the host does not possess the necessary specific food or else the avidity of the cancer for this food is not strong enough to deprive the normal tissue cells of it. When the organism of the host is immunized by treatment with a cell emulsion, these cells bind the specific food, and consequently the cancer cells inoculated subsequently do not find the necessary nourishment and die. Immunity then to cancer growth consists in the lack of food-*athrepsia*.

The experimental evidence adduced by Ehrlich in support of his theory consists in the so-called *Zig-Zag Transplantations, second inoculation on a tumor bearing animal* and *inoculation of an emulsion consisting of two tumors* (carcinoma and sarcoma).

In the present investigation a separate detailed study was made of each of the above-mentioned experimental proofs, and in the following pages each of them will be considered separately. For the present investigation rats and mice were used, and in all five different tumors served for the experiments. Two of these were rat tumors; an extremely virulent Ehrlich sarcoma, and the Flexner-Jobling carcinoma which is not as virulent as the former. The three mice tumors used were Ehrlich's carcinoma of the mouse (No. 33), Bashford's carcinoma of the mouse (No. 27) and Ehrlich's sarcoma of the mouse. The last-named was the most virulent of the mice tumors.

Panimmunity.

Ehrlich did not consider the phenomenon of panimmunity a direct proof of athrepsia, but there must undoubtedly be a certain relationship between the general non-specific character of the former and athrepsia, which is merely a phase in intracellular nutrition. Indeed, if athrepsia is to have a general application, it must be capable of explaining the conditions of panimmunity against the different tumors used in this research, and it may be stated that, in a general way, the fact of panimmunity was found to be correct for the majority of cases. But there were factors observed in the course of these experiments which cannot be explained on the basis of the theory of athrepsia. Of such experiments the following instances may be cited:

Experiment 1.—Twenty-nine rats which had been previously immunized against an inoculation of a rat carcinoma (Flexner-Jobling) were inoculated four weeks later with a rat sarcoma (Ehrlich); of these rats, 23 remained immune and only 6 rats, or 20 per cent. of the inoculated animals, grew the tumor, while of 20 control rats 17, or 85 per cent., took the tumor.

Of these two tumors the Flexner-Jobling carcinoma grows much slower than the Ehrlich rat sarcoma and does not reach so large a size. Consequently the former tumor has less avidity for the specific food than the sarcoma, and does not, according to the Ehrlich theory, use up all this food of the host. Therefore a sufficient amount of specific food should be left for the subsequently inoculated sarcoma cells, and it should be possible to inoculate the latter successfully in all the treated animals.

The following experiment shows again that all the facts of panimmunity cannot be explained on the basis of athrepsia:

Experiment 2.—Twenty rats were inoculated with a mouse sarcoma, and four weeks later when the grafts were completely absorbed were inoculated with a rat sarcoma. Of the 15 rats which survived at the last examination the tumor grew in only 2. This shows 13 per cent. takes, while in the 20 control animals the tumor took in 90 per cent.

It will be shown later that, according to the theory of athrepsia, the cells of a mouse tumor are unable to anchor the specific food of the rat. Consequently the specific food needed for the success of the inoculation of a rat sarcoma should exist in the same quantity in the rats treated with mouse sarcoma as in the control animals; still the rats treated with mouse tissue were immune to the inoculation of the rat tumor. The fact that it is possible to immunize a rat with mouse tissue and vice-versa was previously observed by Carl Lewin and by one of the present writers.

Another factor observed in the course of these experiments on panimmunity, which is of great importance in the consideration of the significance of athrepsia, consists in the following: whenever a tumor succeeds in growing in one of the previously treated animals, it attains the same size and virulence as in the control animals.

This phenomenon indicates that panimmunity with its nonspecific character is in reality an immunity to tissue grafting. On the other hand, when the cancer cells are ingrafted, such preliminary treatment of the animals with living tissue, as is used in experiments on panimmunity, does not exert any influence

whatsoever on the subsequent growth or virulence of the developing tumor. The truth of this assertion is also evident from the fact that no immunization with living tissue is successful if it is done subsequently to the inoculation of the tumor.

The experiments of Rous[2] with inoculation of embryonic tissue show that by the same method an animal may be immunized against grafting of embryonic cells. The panimmunity is in reality an immunity against implantation of tissue. But since in all experimental cancer work with the inoculable tumors the first step always consists in the inoculation of a piece of the tumor, such tissue immunity obscures the actual immunity to the growth and development of the cancer.

Zig-Zag Transplantations.

This phenomenon which served as the main experimental basis for the formation of the theory of athrepsia consists in the following: When a mouse sarcoma is inoculated into a rat it grows there normally for about 8 or 10 days, then ceases to grow and is absorbed. But if before the absorption takes place the nodule is removed from the rat and transferred to a mouse, it continues to grow normally and may be subsequently transferred to another rat, where it will grow for about 8 days and so on.

Ehrlich explains the result of these experiments by the supposition that tumor cells, in order to proliferate, must obtain a certain specific food X, which they can find only in an animal of the same species as that from which the tumor cells have come. Cells of a mouse tumor inoculated into a rat proliferate for a few days, as long as the food X, which they brought over from the mouse, lasts. When this is used up, they do not find this specific food in the rat and cease to grow, but grow again when transferred to a mouse, where they find this specific food X. On the basis of this supposition Ehrlich created the general theory that whenever cancer cells fail to proliferate, it means that they

[2]Rous: *Journ. Exp. Med.*, 1910, xii, 344.

fail to obtain the food X, either because the normal body cells have greater avidity to this food than the cancer cells, or else the cells with which the animals were immunized anchored all the specific food and the cancer cells inoculated subsequently could not obtain it.

The experiments of Zig-Zag Transplantation were undertaken by the writers on all the tumors used in this investigation.

In the course of these experiments certain facts were observed which indicate that athrepsia cannot elucidate all the phenomena observed with Zig-Zag Transplantations.

The inoculation of the rat sarcoma and rat carcinoma into mice and of the Ehrlich's mouse carcinoma into rats uniformly produce small nodules in the host of different species. When these nodules were re-transferred into animals of the species, from which the original tumors were derived, for instance when a rat sarcoma was re-transplanted from a mouse into a rat, not a single good tumor growth could be produced by any of the three above-mentioned tumors.

With Ehrlich's mouse sarcoma and Bashford's mouse carcinoma the results of the experiments appeared to be more successful, *i. e.*, when a nodule of the mouse sarcoma or carcinoma after a sojourn of 8 to 10 days in a rat was removed and inoculated into a mouse, it grew in a certain number of cases into a good-sized tumor. The number of such successful inoculations, however, was much smaller than the number of takes in control animals, *i. e.*, in animals that were inoculated with tumor material which was taken directly from a mouse. While in the former the per cent. of takes was from 15 to 25, the tumors took in 90 per cent. in the control animals.

An analysis of these experiments on Zig-Zag Transplantations shows that all tumors, or at least all the tumors with which this investigation was conducted can be successfully grafted on the phylogenetically closely related animals, *i. e.*, from rat to mouse and vice-versa. The same holds true, according to Rous, for embryonic tissue. But the ingrafted cancer cells do not proliferate indefinitely and become malignant in the host of a for-

eign species. The explanation given by Ehrlich of this phenomenon does not seem to be adequate to explain all the factors in connection with it. If a mouse tumor fails to grow in the rat only for lack of specific food, then it should retain its normal virulence when re-transferred into a host of its own species. But experiments show that the virulence of a mouse or rat tumor is so diminished by its 8 to 10 days' sojourn in a host of a different species that it either fails to take at all when re-inoculated into a host of its own species, or else it takes in a very small percentage. It is evident, then, that the organism of the temporary host has a decided noxious affect upon the proliferating activities of the tumor cells; and, moreover, while the capacity for ingrafting in an animal of a foreign species is common to all the tumors studied, is non-specific, the amount of injury sustained through the sojourn in the foreign host is different with the different tumors.

Second Inoculation on a Tumor Bearing Animal.

Ehrlich maintains that animals on whom a successful inoculation of another tumor was previously made appeared to be immune against a subsequent inoculation, either of the same or another tumor. The opposite results found by Hertwig and Poll,[3] Gierke,[4] and Borrel,[5] he explains by the fact that in their experiments they used for the primary inoculations tumors of low virulence and slow growth, while in his experiments the animals received for the first inoculation an extremely virulent tumor. Only a very virulent rapidly growing tumor anchors all the specific food X, and consequently makes the successful take of the subsequently inoculated tumor impossible. A slow-growing tumor leaves a sufficient amount of the specific food for the successful take of a subsequent inoculation.

[3]Hertwig and Poll: *Abhand. d. K. preuss. Akad. d. Wissensch.*, 1907, I, *Abhand.* I.

[4]Gierke: *Betir. z. Path. Anat. u. z. allg. Path.*, 1908, xliii, 328.

[5]Borrel: *Bull. de l'Inst. Pasteur*, 1907, v, 207.

Experiments with a double inoculation were conducted with Flexner-Jobling rat carcinoma and Ehrlich's rat sarcoma. The latter is an extremely virulent and rapidly growing tumor and still a subsequent inoculation of the carcinoma was just as successful on an animal bearing a large sarcoma as the inoculation of the sarcoma on an animal with a growth of carcinoma. The most plausible explanation of the discrepancies in similar experiments of different investigators consists in the different behavior of the different tumor cells after a successful grafting. This latter factor is most evident in the series of experiments with an inoculation of an emulsion consisting of two tumors.

Inoculation of an Emulsion Consisting of Two Tumors.

Apolant inoculated an emulsion of a carcinoma and sarcoma into an animal and developed a mixed tumor showing the structure both of the carcinoma and sarcoma. If one of the tumors was originally more virulent than the other, or its virulence was increased artificially, then the resulting growth showed the structure of the more virulent tumor only. Ehrlich adduces the results of this experiment as another proof of the fact that the more virulent tumor cells obtain all the specific food of the host, causing the cells of the weaker tumor to die of starvation.

These experiments were repeated with two emulsions. One, consisting of the cells of Flexner-Jobling carcinoma and of Ehrlich's sarcoma of the rat, was injected into rats; and another, consisting of Bashford's carcinoma and Ehrlich's mouse sarcoma, was injected into mice. In both instances the sarcoma was a great deal more virulent than the carcinoma. The relationship in the virulence as between Flexner carcinoma and Ehrlich's sarcoma of the rat, and Bashford carcinoma and Ehrlich sarcoma of the mouse, is quite identical, but a most surprising difference was observed in the results of the two experiments. While in the rats on the injection of the emulsion there developed a mixed tumor, showing the structure both of carcinoma and sarcoma, the tumor in the mice appeared to be pure sarcoma.

Thus it seems that different tumors behave in a different manner when introduced simultaneously into an organism.

Of all the experiments stated above, the Zig-Zag Transplantations undoubtedly offer the best field for the analysis of the different phenomena of immunity in cancer. The apparent success of an inoculation of a mouse tumor into a rat may mean only the success of the initial grafting, since a rat possesses a congenital racial immunity against the growth of a mouse cancer. Indeed the nodule which develops in the rat upon an inoculation of a mouse tumor never develops into a large malignant growth. There is, consequently, a condition here, in which the dual character of the immunity to an inoculable tumor is perfectly apparent. The rat is susceptible to the grafting of a mouse sarcoma, but immune against its malignant development.

The success of the grafting of the inoculated cells undoubtedly depends upon conditions of nutrition. Thus *athrepsia* may serve to explain the conditions of resistance and susceptibility to the grafting of tumor, as well as tissue, cells. The formation of a highly vascularized stroma around the successfully ingrafted tissue and the lack of stroma formation in an unsuccessful graft (Russell[6]) also shows the importance of nutrition during this phase of the inoculation of a tumor.

On the other hand, the same experiments on Zig-Zag Transplantations clearly indicate that differences in nutrition cannot explain the reason why, in one successful tumor graft, a small nodule will form which is subsequently absorbed, while in another the nodule will grow into a large tumor and kill the animal.

As stated above, this investigation has shown that a lack of specific food alone cannot explain the failure of a mouse carcinoma or sarcoma to develop into a malignant *tumor* in the rat. The organism of the rat has a direct influence upon the mouse tumor, and its virulence is greatly diminished after a sojourn of a few days in this animal. The organism of the rat had, con-

[6] Russell: *Scientific Reports of the Imperial Cancer Research Fund*, 1908, No. 3, 341.

sequently, a noxious affect upon the proliferating activities of the cells of the mouse tumor. It is of great interest to note, in this connection, that upon an inoculation of these weakened cells into another generation of mice the normal virulence of the former returns. The following series of experiments illustrates this point:

Experiment 3.—Twenty mice were inoculated with pieces of the Ehrlich mouse sarcoma taken from rats. Nineteen mice survived at the last examination, and of these one mouse took the tumor, which presents 5 per cent. of takes. The tumors from this mouse was inoculated in 20 other mice and the tumor took in 14 mice, or in 70 per cent.

It appears from these experiments that a mouse tumor through a sojourn in the rat reacquires the characteristics of a spontaneous tumor. Namely, it takes in a small percentage of inoculated animals, and this percentage increases in subsequent generations. But the diminution of the virulence of the tumor which takes place in the organism of the rat is due to congenital racial immunity of the latter. Furthermore, the immunity of the rat against mouse tumor is not due to conditions of nutrition, but is accompanied by an active noxious influence of the organism of the rat on the mouse tumor. Thus indirect evidence is brought forward through this investigation to show that immunity to cancer growth (but not to tumor grafting) is caused not by differences in intercellular nutrition, but by an active inhibitory influence of the organism of the host on the cancer cells. The search for direct evidence and the true nature of this inhibitory influence is the subject of our further studies on immunity in cancer of the white rat.

Discussion.

Dr. Rous: I have been somewhat interested in the subject of athreptic immunity, not directly, but indirectly through some experiments done for another purpose.

Some two years ago Gay, working for the Cancer Fund at Harvard, published a paper on Immunity to Cancer in which he divided the growth of cancer into two stages—one pre-meta-

static, the other metastatic. He said that if, during the pre-metastatic period a second graft of tumor were made, the first tumor frequently retrogressed and the second usually failed to develop. Later, when metastasis was occurring, the course of the primary tumor was not affected by the second graft, and this, too, grew.

Interested by the work, I made some experiments which have not yet been reported. The Flexner-Jobling adeno-carcinoma of the rat was used, the same growth employed by Gay. My results do not confirm his. They give, however, some data on the subject of athreptic immunity. Here was a large, rapidly growing tumor and one could observe its influence on a second graft. But no influence was observed. Both in the period before metastasis and later, the second graft grew with the same rapidity as in the control animal. These observations form evidence against the current theory of athrepsia. In this connection it is to be remembered that when the primary tumor is very large, one is dealing with a sick, emaciated animal such as one would expect to find a poor host for new grafts. The failure of such grafts can certainly be explained from other points of view than that of athrepsia.

Dr. MacCallum was especially struck by the statement that the growth of pieces of mouse tumor, after a sojourn in a rat, when again transplanted to mice, showed a diminished percentage of "takes." This, if true, seemed distinctly opposed to the athrepsia idea. It might perhaps be thought that an explanation would be found in some fault of technique; but that seems hardly a valid objection, in view of the care that was taken in these experiments. The inoculation of mixed emulsions of tumors had certainly produced the most beautiful combinations of sarcoma and carcinoma.

THE EXHIBITION OF PHAGOCYTOSIS BY TUMOR CELLS.

F. M. HANES, M.D.

When the transplantable sarcomas and carcinomas of rats and mice are cultivated outside the body, the cells wander from the original piece of tissue by amœboid movement. Although the amœboid movements of tumor cells growing in vitro are slower than those of the amœba, they yet show numerous pseudopods which enlarge and retract in typical fashion.

The wandering amœboid tumor cells show active phagocytosis. If carmine particles are placed in the plasma used for cultivating the tumors, the wandering tumor cells take up the carmine granules most actively. It is thus seen that metazoan fixed cells, when placed in a favorable environment for growth outside the body, behave like some protozoan cells, showing amœboid movement and phagocytosis.

Discussion.

Dr. MacCallum thought that care must be exercised in comparing the growth of tumors in vitro with those in the body, for it is quite conceivable that tissues from the benign tumor would show the same wandering or amœboid activity as those from malignant tumors.

The time during which one can observe the characteristics of growth of tumor cells in vitro is so short that it hardly gives us an opportunity to observe the development of the peculiarities of malignant tumors. We have still, as the chief problem, the explanation of the difference inherent in the cells which gives them their enormous energy of growth, quite in contrast with that of normal cells.